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CSL LIMITED

PROVISIONAL SPECIFICATION

Invention Title:

Synthetic peptides containing protective epitopes for the treatment and prevention of periodontitis associated with porphyromonas gingivalis

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PROVISIONAL SPECIFICATION

Invention Title:

Synthetic peptides containing protective epitopes for the treatment and prevention of periodontitis associated with porphyromonas gingivalis

The invention is described in the following statement:

Peptides for Treatment and/or Prevention of Periodontitis

FIELD OF THE INVENTION

This invention relates to an oral composition and an immunogenic composition for the suppression of the pathogenic effects of the intra-oral bacterium *Porphyromonas gingivalis* associated with periodontal disease. It also relates to diagnostic tests for the presence of *Porphyromonas gingivalis* in subgingival plaque samples and specific antibodies against *P. gingivalis* antigens in sera. The compositions comprise synthetic peptide constructs corresponding to protective epitopes of the PrtR-PrtK proteinase-adhesin complex of *Porphyromonas gingivalis*. The synthetic peptide constructs are useful as immunogens in vaccine formulations for active immunization and can be used to generate protein-specific and peptide-specific antisera useful for passive immunization and as reagents for diagnostic assays.

BACKGROUND OF THE INVENTION

Periodontal diseases are bacterial-associated inflammatory diseases of the supporting tissues of the teeth and range from the relatively mild form of gingivitis, the non-specific, reversible inflammation of gingival tissue to the more aggressive forms of periodontitis which are characterised by the destruction of the tooth's supporting structures. Periodontitis is associated with a subgingival infection of a consortium of specific Gram-negative bacteria that leads to the destruction of the periodontium and is a major public health problem. One bacterium that has attracted considerable interest is *Porphyromonas gingivalis* as the recovery of this microorganism from adult periodontitis lesions can be up to 50% of the subgingival anaerobically cultivable flora, whereas *P. gingivalis* is rarely recovered, and then in low numbers, from healthy sites. A proportional increase in the level of *P. gingivalis* in subgingival plaque has been associated with an increased severity of periodontitis and eradication of the microorganism from the cultivable subgingival microbial population is accompanied by resolution of the disease. The progression of periodontitis lesions has been demonstrated in monkey, rats and mice with the subgingival implantation of *P. gingivalis*. These findings in both animals and humans suggest a major role for *P. gingivalis* in the development of adult periodontitis.

P. gingivalis is a black-pigmented, anaerobic, proteolytic Gram-negative rod that obtains energy from the metabolism of specific amino acids. The

Peptides (EP1-EP7) of Table 1 represent sequences of the adhesin domains of the PrtR and PrtK protein-adhesin complex.

Accordingly, in a first aspect the present invention consists in a composition for use in raising an immune response against *Porphyromonas gingivalis*, the composition comprising a suitable adjuvant and/or acceptable carrier or excipient and at least one peptide of not more than 50 amino acids which peptide includes at least one *P. gingivalis* epitope, the at least one *P. gingivalis* epitope being selected from the epitopes included within a peptide selected from the group consisting of:

EP1 FLLDADHNTFGSVIPATGPLFTGTASS,
EP2 LYSANFESLIPANADPVVTTQNIIVTG,
EP3 LYSANFEYLIPANADPVVTTQNIIVTG,
EP4 TNPEPASGKMWIAGDGGNQP,
EP5 RYDDFTFEAGKKYTFTMRRAGMGDGTD,
EP6 DDYVFEAGKKYHFLMKKMGSGDGTE, and
EP7 TNPEPASGKMWIAGDGGNQPARYDDFTFEAGKKYTFTMRRAG MGDGTD.

Where the composition includes more than one peptide the peptides may be present in the composition as individual peptides or in multimeric forms. Where multimeric forms are used the multimer may comprise multiple copies of the same peptide, however, it is preferred that the multimer includes different peptides. Peptide multimers may be prepared as described in PCT/AU98/00076, the entire contents of which are incorporated herein by reference.

In a second aspect of the present invention consists in a peptide, the peptide having not more than 50 amino acids which peptide includes at least one *P. gingivalis* epitope, the *P. gingivalis* epitope being selected from the epitopes included in the peptides selected from the group consisting of:

EP1 FLLDADHNTFGSVIPATGPLFTGTASS
EP2 LYSANFESLIPANADPVVTTQNIIVTG
EP3 LYSANFEYLIPANADPVVTTQNIIVTG
EP4 TNPEPASGKMWIAGDGGNQP
EP5 RYDDFTFEAGKKYTFTMRRAGMGDGTD
EP6 DDYVFEAGKKYHFLMKKMGSGDGTE
EP7 TNPEPASGKMWIAGDGGNQPARYDDFTFEAGKKYTFTMRRAG MGDGTD

As will be readily apparent to persons skilled in this area these antibodies may be used in diagnostic tests or in pharmaceutical formulations.

In a fourth aspect the present invention consists in a method of reducing the prospect of *P. gingivalis* infection in an individual and/or severity of disease, the method comprising administering to the individual an amount of the composition of the first aspect effective to induce an immune response in the individual directed against *P. Gingivalis*.

In a fifth aspect the present invention consists in a method of reducing the prospect of *P. gingivalis* infection in an individual and/or severity of disease, the method comprising administering to the individual an effective amount of an antibody of the third aspect.

Peptides can be synthesized using one of the several methods of peptide synthesis known in the art including standard solid phase peptide synthesis using t-butyloxycarbonyl amino acids (Mitchell et al., 1978, J. Org. Chem. 43:2845-2852) using 9-fluorenylmethyloxycarbonyl (Fmoc) amino acids on a polyamide support (Druland et al., 1986, J. Chem. Soc.Perkin Trans. 1 125-137) by pepscan synthesis (Geysen et al., 1987, J. Immunol Methods 03:259; 1984, Proc. Natl. Acad. Sci. USA, 81:3998) or by standard liquid phase synthesis.

A variety of methods for the synthesis of multivalent/multipeptide high molecular weight peptide molecules can be used to synthesize the peptide antigens. This will be achieved using known in the art and novel ligation strategies.

Peptides from Table 1 can be synthesized in such a way as to contain two ligands, which can be the same or different, which may or may not be the complementary ligand. These bi-modal peptides can incorporate any ligand thus linkages such as thioether, thioester, hydrazone, oxime, thiazolidine can be utilised for the synthesis of multipeptide constructs Shao and Tam., 1995, J. Am. Chem. Soc. 117, 3893-3899, Rose, et al 1996, Bioconjugate Chem. 7(5):552-556, Rose. K., 1994, J. Am. Chem. Soc. 116:30-33, Canne., et al 1995, J. Am. Chem. Soc. 117:2998-3007, Lu., et al, 1991, Mol. Immunol 28(6):623-630, Liu and Tam., 1994, Proc. Natl. Acad. Sci. 91,:6584-6588. A novel ligating strategy is to use the known reaction between thioanisole and acryloyl peptides (O'Brien-Simpson et al., 1997, J. Am. Chem. Soc. 119 (6) which results in the para substitution of thioanisole by the double bond in acidic conditions. By synthesising and mixing

in aqueous solvents similar to those used for solution phase ligation except that separation of the ligand product from unreacted bi-modal peptide can be achieved by simply washing the solid support. The reaction can be monitored by ninhydrin or trinitrobenzene sulphonic acid tests, where by, lysine residues within the bi-modal peptide would need to be protected eg. with (4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl (Dde) which is stable to acid cleavage but can be removed with hydrazine. Figure 3 shows the ligation strategy for the solid phase.

Bi-modal peptides can be synthesized so that ligands are at the N- and C- terminus. This would allow the preparation of cyclic peptides and the formation of di-peptide constructs where by peptides can run parallel or anti parallel to each other by either coupling N- to N- and C- to C- termini or N- to C- termini together respectively (Figure 4).

Another technique for the synthesis of multivalent peptide constructs is to ligate peptides on to an oligolysine support (Rose, et al 1996, Bioconjugate Chem. 7(5):552-556, Canne., et al 1995, J. Am. Chem. Soc. 117:2998-3007 and Lu., et al, 1991, Mol. Immunol 28(6):623-630). By incorporating a number of different ligands and or protected ligands on to the lysine support, peptides can be ligated to a particular position on the support. Ligation chemistries such as oxime or hydrazone with haloacylation and Friedal-Craft alkylation can be used sequentially without the need for ligand protection. Ligand protection can be used to increase the number of different peptides incorporated on to the lysine support. Figure 5 demonstrates the synthesis protocol.

Another method known in the art is the synthesis of acryloyl peptides and their polymerisation with acrylamide (O'Brien-Simpson et al., 1997, J. Am. Chem. Soc. 119 (6)) or acryloyl amino acids. Peptides from the PrtR-PrtK protein complex listed in Table 1 can be acryloylated and polymerised either singularly or in combination. Although this method allows the polymerisation of a number of peptides together the order in which peptides are incorporated can not be controlled.

The final peptide construct may or may not contain all, sum or part of the peptides listed in Table 1. Also the construct may or may not contain promiscuous T-cell epitopes known in the art (Kaumaya et al 1994, in Solid Phase Synthesis, Ed Epton, R) or a derived sequence from structural/binding motifs of MHC class II binding peptides (O'Sullivan et al., 1991, J. Immunol,

block copolymers; detergents such as Tween®-80; Quil® A, mineral oils such as Drakeol or Marcol, vegetable oils such as peanut oil; *Corynebacterium*-derived adjuvants such as *Corynebacterium parvum*; *Propionibacterium*-derived adjuvants such as *Propionibacterium acne*; *Mycobacterium bovis* (Bacille Calmette and Guerin or BCG); interleukins such as interleukin 2 and interleukin 12; monokines such as interleukin 1; tumour necrosis factor; interferons such as gamma interferon; combinations such as saponin-aluminium hydroxide or Quil-A aluminium hydroxide; liposomes; ISCOM adjuvant; mycobacterial cell wall extract; synthetic glycopeptides such as murarmyl dipeptides or other derivatives; Avridine; Lipid A derivatives; dextran sulfate; DEAE-Dextran or with aluminium phosphate; carboxypolymethylene such as Carbopol' EMA; acrylic copolymer emulsions such as Neocryl A640 (e.g. U.S. Pat. No. 5,047,238); vaccinia or animal poxvirus proteins; sub-viral particle adjuvants such as cholera toxin, or mixtures thereof.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Synthesis of Bi-modal Peptides Although a specific example is shown here any ligand can be introduced at the a or e amino groups of lysine. (a) acylation e.g. amino acid:HOBt:HBTU:DIPEA 1:1:1:1.5 in dimethyl formamide (DMF). (b) Fmoc deprotection e.g. 20% piperidine in DMF. (c) Levulinic acid: diisopropyl carbodiimide (DIC) 2:1 in dichloromethane (DCM), 1h. (d) Mtt removal, 3x 1% TFA in DCM, 3 mins. (e) Fmoc-Hydrazino benzoic acid : DIC 2:1, in DCM, 1h. (f) Acid cleavage e.g. TFA : water 95:5.

Figure 2: Synthesis of multivalent peptide constructs using bi-modal peptides. (a) Ligation. 8 M urea and 0.1 M NaH_2PO_4 (pH range 3-4.7). Ligation can be monitored by reverse phase analytical HPLC and mass spectrometry. (b) Deprotection, e.g. Aloc is removed by palladium(0)-catalyzed allyl group transfer to a basic receptor. The ligation product can be purified by preparative HPLC and lyophilised. (c) Ligation. Similar conditions as described in (a). Different ligation chemistries can be used by synthesising peptides with different ligands and synthesising non-

of peptides can be ligated onto a single multiple antigenic peptide. The example shown is of peptides listed in Table 1. (a) Ligation, 95% aqueous TFA. . Ligation can be monitored by reverse phase analytical HPLC and mass spectrometry. Deprotection, Aloc can removed by palladium(0)-catalyzed allyl group transfer to a basic receptor. after purifaction the second peptide can be ligated on to the MAP, (c) 8 M urea and 0.1 M NaH_2PO_4 (pH range 3-4.7).

Figure 6: Immunoblot analysis of PrtR-PrtK protein complex with human and mouse sera. PrtR-PrtK proteins were probed with sera from patient D20, patient D24, patient H10 and anti-PrtR-PrtK sera from BALB/c mice. Immunoblot analysis was carried out as per Example 1. Molecular weight markers are shown as kDa.

Figure 7: Serum IgG antibody responses assessed by ELISA to Porphyromonas gingivalis PrtR-27 overlapping peptides. Twenty one PIN-bound peptides were probed with normal mouse sera (■), protective mouse sera (□), normal human serum (□), patient D24 sera (□), patient H10 sera (□) and patient D20 sera. ELISAs were developed as per Example 1.

DETAILED DESCRIPTION OF THE INVENTION

This invention relates to an oral composition and a vaccine for the suppression of the pathogenic effects of the intra-oral bacterium *Porphyromonas gingivalis* associated with periodontal disease. It also relates to diagnostic tests for the presence of *Porphyromonas gingivalis* in subgingival plaque samples and specific anti-*P. gingivalis* antibodies in sera. The peptide antigens of Table 1 can be synthesized individually or as multimetric or multipeptide constructs.

The synthetic peptide antigens are used to generate polyclonal or monoclonal antibodies using standard techniques. The animals used for antibody generation can be mice, rabbits, goats, chickens, sheep, horses, cows etc. When a high antibody titre against the antigens is detected by immunoassay the animals are bled or eggs or milk are collected and the serum prepared and/or antibody purified using standard techniques or monoclonal antibodies produced by fusing spleen cells with myeloma cells using standard techniques. The antibody (immunoglobulin fraction) may be

The pH of such liquid and other preparations of the invention is generally in the range of from about 4.5 to about 9 and typically from about 5.5 to 8. The pH is preferably in the range of from about 6 to about 8.0, preferably 7.4. The pH can be controlled with acid (e.g. citric acid or benzoic acid) or base (e.g. sodium hydroxide) or buffered (as with sodium citrate, benzoate, carbonate, or bicarbonate, disodium hydrogen phosphate, sodium dihydrogen phosphate, etc).

Other desirable forms of this invention, the oral composition may be substantially solid or pasty in character, such as toothpowder, a dental tablet or a dentifrice, that is a toothpaste (dental cream) or gel dentifrice. The vehicle of such solid or pasty oral preparations generally contains dentally acceptable polishing material. Examples of polishing materials are water-insoluable sodium metaphosphate, potassium metaphosphate, tricalcium phosphate, dihydrated calcium phosphate, anhydrous dicalcium phosphate, calcium pyrophosphate, magnesium orthophosphate, trimagnesium phosphate, calcium carbonate, hydrated alumina, calcined alumina, aluminum silicate, zirconium silicate, silica, bentonite, and mixtures thereof. Other suitable polishing material include the particulate thermosetting resins such as melamine-, phenolic, and urea-formaldehydes, and cross-linked polyepoxides and polyesters. Preferred polishing materials include crystalline silica having particle sized of up to about 5 microns, a mean particle size of up to about 1.1 microns, and a surface area of up to about 50,000 $cm^2/gm.$, silica gel or colloidal silica, and complex amorphous alkali metal aluminosilicate.

When visually clear gels are employed, a polishing agent of colloidal silica, such as those sold under the trademark SYLOID as Syloid 72 and Syloid 74 or under the trademark SANTOCEL as Santocel 100, alkali metal alumino-silicate complexes are particularly useful since they have refractive indices close to the refractive indices of gelling agent-liquid (including water and/or humectant) systems commonly used in dentifrices.

Many of the so-called "water insoluble" polishing materials are anionic in character and also include small amounts of soluble material. Thus, insoluble sodium metaphosphate may be formed in any suitable manner as illustrated by Thorpe's Dictionary of Applied Chemistry, Volume 9, 4th Edition, pp. 510-511. The forms of insoluble sodium metaphosphate known as Madrell's salt and Kurrol's salt are further examples of suitable materials.

cellulose, and colloidal silica such as finely ground Syloid (e.g. 244). Solubilizing agents may also be included such as humectant polyols such propylene glycol, dipropylene glycol and hexylene glycol, cellosolves such as methyl cellosolve and ethyl cellosolve, vegetable oils and waxes containing at least about 12 carbons in a straight chain such as olive oil, castor oil and petrolatum and esters such as amyl acetate, ethyl acetate and benzyl benzoate.

It will be understood that, as is conventional, the oral preparations are to be sold or otherwise distributed in suitable labelled packages. Thus, a jar of mouthrinse will have a label describing it, in substance, as a mouthrinse or mouthwash and having directions for its use; and a toothpaste, cream or gel will usually be in a collapsible tube, typically aluminium, lined lead or plastic, or other squeeze, pump or pressurized dispenser for metering out the contents, having a label describing it, in substance, as a toothpaste, gel or dental cream.

Organic surface-active agents are used in the compositions of the present invention to achieve increased prophylactic action, assist in achieving thorough and complete dispersion of the active agent throughout the oral cavity, and render the instant compositions more cosmetically acceptable. The organic surface-active material is preferably anionic, nonionic or ampholytic in nature which does not denature the antibody of the invention, and it is preferred to employ as the surface-active agent a detersive material which imparts to the composition detersive and foaming properties while not denaturing the antibody. Suitable examples of anionic surfactants are water-soluble salts of higher fatty acid monoglyceride monosulfates, such as the sodium salt of the monosulfated monoglyceride of hydrogenated coconut oil fatty acids, higher alkyl sulfates such as sodium lauryl sulfate, alkyl aryl sulfonates such as sodium dodecyl benzene sulfonate, higher alkylsulfo-acetates, higher fatty acid esters of 1,2-dihydroxy propane sulfonate, and the substantially saturated higher aliphatic acyl amides of lower aliphatic amino carboxylic acid compounds, such as those having 12 to 16 carbons in the fatty acid, alkyl or acyl radicals, and the like. Examples of the last mentioned amides are N-lauroyl sarcosine, and the sodium, potassium, and ethanolamine salts of N-lauroyl, N-myristoyl, or N-palmitoyl sarcosine which should be substantially free from soap or similar higher fatty acid material. The use of these sarconite compounds in

about 8, preferably about 6 to 8, for at least 2 weeks up to 8 weeks or more up to a lifetime.

The compositions of this invention can be incorporated in lozenges, or in chewing gum or other products, e.g. by stirring into a warm gum base or coating the outer surface of a gum base, illustrative of which may be mentioned jelutong, rubber latex, vinylite resins, etc., desirably with conventional plasticizers or softeners, sugar or other sweeteners or such as glucose, sorbitol and the like.

Another important form of the invention is a vaccine based on the synthetic peptide antigens and suitable adjuvant delivered by nasal spray, orally or by injection to produce a specific immune response against the antigen thereby reducing colonisation of *P. gingivalis* and reducing virulence thereby preventing disease. Unlike whole *P. gingivalis* cells or other previously prepared antigens, the peptide antigens described herein are safe and effective antigens for the preparation of a vaccine for the prevention of *P. gingivalis*-associated periodontal disease. Additionally, according to the present invention, antigenic peptide produced may be used to generate *P. gingivalis* antisera useful for passive immunization against periodontal disease and infections caused by *P. gingivalis*.

The following examples are further illustrative of the nature of the present invention, but it is understood that the invention is not limited thereto. All amounts and proportions referred to herein and in the appended claims are by weight unless otherwise indicated.

EXAMPLE 1

(i) Identification of Protective Epitopes in the PrtR-PrtK Proteinase-Adhesin Complex

The PrtR-PrtK proteinase-adhesin complex was purified as described previously in International Patent Application No. PCT/AU96/00673 and was shown to confer protection to mice against challenge with *P. gingivalis* when used as a vaccine. The PrtR-PrtK complex was tested in the mouse abscess model. This model is loosely based on the methods of Kesavalu *et al.* (1992) [Infect Immun 60: 1455-1464]. A typical experiment is outlined below. Briefly BALB/c mice were obtained from ARC (Perth, Australia) and were immunised subcutaneously in the scruff of the neck with the preparations and doses according to Table 2 before challenge with live *P. gingivalis* strain

challenge dose at a single site in the middle of the back. A 0.1 ml dose was given representing a predicted challenge dose of 3×10^9 bacteria per mouse. The inoculum dose was confirmed by culturing various dilutions of the challenge dose on lysed HBA plates and examining the number of colonies 7 days later.

Following challenge mice were examined daily for the number and size of lesions on their body and their size estimated by measuring the approximate surface area in mm^2 involved. Previous experiments had shown that in unimmunized mice, lesions developed on the belly of the mice following inoculation of live bacteria into the back or side. Any distressed animals were culled. Observations were carried out over two weeks and a summary of one such experiment is summarised below in Table 3. In this experiment while a dose of 3×10^9 bacteria per mouse was the desired number of bacteria, after plating out of the inoculum it was calculated that each mouse actually received a challenge dose of 3.17×10^9 live *P. gingivalis* bacteria strain W50.

When mice were immunised significant reductions ($p < 0.005$) were seen in the size of the lesions with whole formalin killed *P. gingivalis* strain W50 cells (Group 1), and the PrtR-PrtK complex (Group 2) when compared with the lesion size of the animals receiving FIA (Group 3) (Table 3). These results clearly show that the PrtR-PrtK complex works effectively as an immunogen. The only group of animals that had a number of animals (40%) that exhibited no visible lesions at all was the PrtR-PrtK complex group (Group 2). All other groups, including formalin killed cells (Group 1), had all animals exhibiting visible lesions indicating that the PrtR-PrtK complex was a better immunogen than formalin killed cells.

anti-human IgG horse radish peroxidase conjugate or anti-mouse IgG horse radish peroxidase conjugate. After washing (4x TN buffer containing 0.05% v/v Tween 20) bound antibody was detected with 2.8 M 4-chloro-1-napthol in TN buffer containing 16.6% v/v methanol and 0.05% v/v of a 30% H_2O_2 solution. Colour development was stopped by rinsing the membranes with Milli Q water.

A protein band at 44kDa was shown to react with all of the sera tested (Figure 6). The protective mouse sera and sera from patient H10 who does not have periodontitis also bound to a protein band of 27kDa (PrtR27 adhesin). Patient sera from D20 (advanced periodontitis) did not react with this 27 kDa protein suggesting that antibodies directed toward the 27kDa adhesin may have provided protection against peridontitis in patient H10 and the immunoprotected mice.

Epitope Mapping analysis

Twenty overlapping 13mer peptides (overlay by 6 and offset by 7 residues) corresponding to the N-terminal 148 residues of the PrtR27 were synthesised by Chiron Technologies (Melbourne, Australia) using the multipin peptide synthesis system. The sequence of the N-terminal 148 residues of the PrtR27 adhesin is as follows:

ANEAKVVLAADNVWDGNTGYQFLLDADHNTFGSVIPATGPLFTGTASSDLYSA
NFESLIPANADPVVTTQNIIVTGQGEVVIPGGVYDYCITNPEPASGKMWIAGD
GGNQPARYDDFTFEAGKKYTFTMRRAGMGDGTDMEVEDDSPA

The overlapping peptides synthesised were:

ANEAKVVLAADNV
LAADNVWDGNTGY
DGNTGYQFLLDAD
FLLDADHNTFGSV
NTFGSVIPATGPL
PATGPLFTGTASS
TGTASSDLYSANF
LYSANFESLIPAN
SLIPANADPVVTT
DPVVTTQNIIVTG
NIIVTGQGEVVIP
GEVVIPGGVYDYC

LYSANFEYLIPANADPVVTTQNIIVTG. The PrtR44 sequence homologous to the PrtR27 protective epitope is DDYVFEAGKKYHFLMKKMGSGDGTE.

These sequences incorporated into a peptide construct or peptide protein conjugate therefore could form the basis of an immunogen to provide protection against periodontitis.

(ii) Synthesis of Peptide Antigens and Multiple Constructs

The peptides of Table 1 (EP1-EP7) can be synthesized using standard Fmoc or tBoc synthesis strategies and multipeptide constructs can be synthesized using the strategies outlined in Figs. 1-5.

(iii) Preparation of Antibodies

Serum antibodies can be obtained by immunising horses, rabbits, sheep or dairy cows.

Immunizations can be carried out using standard procedures. The initial immunisation is usually with a mixture of the antigen and Freund's incomplete adjuvant. The antibodies can be recovered from the animals serum or milk using standard procedures.

EXAMPLE 2

Methods for using antigenic peptides in diagnostic immunoassays.

The *P. gingivalis* peptide antigens described herein can be synthesized for use as immunogens in vaccine formulations; and as antigens for diagnostic assays or for generating *P. gingivalis*-specific antisera of therapeutic and/or diagnostic value.

The peptides disclosed in Table 1 can be synthesized individually or chemically-linked using the strategies of Figs. 1 - 5. The peptides can be synthesized using one of the several methods of peptide synthesis known in the art including standard solid phase peptide synthesis using tertbutyloxycarbonyl amino acids (Mitchell *et al.*, 1978, *J. Org. Chem.* 43:2845-2852), using 9-fluorenylmethyloxycarbonyl amino acids on a polyamide support (Dryland *et al.*, 1986, J. *Chem. So. Perkin Trans.* I, 125-137); by pepscan synthesis (Geysen *et al.*, 1987, *J. Immunol. Methods* 03:259; 1984, *Proc. Natl. Acad. Sci. USA* 81:3998); or by standard liquid phase peptide synthesis. Modification of the peptides or oligopeptides, such

In one illustration of the invention, the dipeptide EP4-EP5 construct having the properties desirable of a vaccine antigen, the dipeptide construct can be synthesized using the method described herein in Figs. 1-5.

The synthetic peptide is included as the relevant immunogenic material in the vaccine formulation, and in therapeutically effective amounts, to induce an immune response. Many methods are known for the introduction of a vaccine formulation into the human or animal to be vaccinated. These include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, ocular, intranasal, and oral administration. The vaccine may further comprise a physiological carrier such as a solution, a polymer or liposomes; and an adjuvant, or a combination thereof.

Various adjuvants are used in conjunction with vaccine formulations. The adjuvants aid by modulating the immune response and in attaining a more durable and higher level of immunity using smaller amounts of vaccine antigen or fewer doses than if the vaccine antigen were administered alone. Examples of adjuvants include incomplete Freund's adjuvant (ISA), Adjuvant 65 (containing peanut oil, mannide monooleate and aluminum monostrearate), oil emulsions, Ribi adjuvant, the pluronic polyols, polyamines, Avridine, Quil A, saponin, MPL, QS-21, and mineral gels such as aluminum hydroxide, aluminum phosphate, etc.

Another embodiment of this mode of the invention involves the production of antigen-specific amino acid sequences as a hapten, i.e. a molecule which cannot by itself elicit an immune response. In such case, the hapten may be covalently bound to a carrier or other immunogenic molecule which will confer immunogenicity to the coupled hapten when exposed to the immune system. Thus, such a antigen-specific hapten linked to a carrier molecule may be the immunogen in a vaccine formulation.

As an alternative to active immunization, immunization may be passive, i.e. immunization comprising administration of purified immunoglobulin containing antibody against synthetic peptides.

EXAMPLE 6

The following is a proposed toothpaste formulation.

Ingredient	% w/w
Dicalcium phosphate dihydrate	50.0
Sorbitol	10.0
Glycerol	10.0
Sodium carboxymethyl cellulose	1.0
Lauroyl diethanolamide	1.0
Sucrose monolaurate	2.0
Flavour	1.0
Sodium saccharin	0.1
Sodium monofluorophosphate	0.3
Chlorhexidine gluconate	01
Dextranase	0.01
Bovine milk Ig containing anti-peptide Abs	0.1
Water	balance

EXAMPLE 7

The following is a proposed toothpaste formulation.

Ingredient	% w/w
Sorbitol	22.0
Irish moss	1.0
Sodium Hydroxide (50%)	1.0
Gantrez	19.0
Water (deionised)	2.69
Sodium Monofluorophosphate	0.76
Sodium saccharine	0.3
Pyrophosphate	2.0
Hydrated alumina	48.0
Flavour oil	0.95
anti-peptide mouse monoclonal	0.3
sodium lauryl sulphate	2.00

EXAMPLE 10

The following is a proposed mouthwash formulation.

Ingredient	% w/w
Gantrez S-97	2.5
Glycerine	10.0
Flavour oil	0.4
Sodium monofluorophosphate	0.05
Chlorhexidine gluconate	0.01
Lauroyl diethanolamide	0.2
Mouse anti-peptide monoclonal	0.3
Water	balance

EXAMPLE 11

The following is a proposed lozenge formulation.

Ingredient	% w/w
Sugar	75-80
Corn syrup	1-20
Flavour oil	1-2
NaF	0.01-0.05
Mouse anti-peptide monoclonal	0.3
Mg stearate	1-5
Water	balance

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CLAIMS:-

1. A composition for use in raising an immune response against *Porphyromonas gingivalis*, the composition comprising a suitable adjuvant and/or acceptable carrier or excipient and at least one peptide of not more than 50 amino acids which peptide includes at least one *P. gingivalis* epitope, the at least one *P. gingivalis* epitope being selected from the epitopes included within a peptide selected from the group consisting of:
FLLDADHNTFGSVIPATGPLFTGTASS,
LYSANFESLIPANADPVVTTQNIIVTG,
LYSANFEYLIPANADPVVTTQNIIVTG,
TNPEPASGKMWIAGDGGNQP,
RYDDFTFEAGKKYTFTMRRAGMGDGTD,
DDYVFEAGKKYHFLMKKMGSGDGTE, and
TNPEPASGKMWIAGDGGNQPARYDDFTFEAGKKYTFTMRRAGMGDGTD.

2. A composition as claimed in claim 1 in which the at least one peptide comprises a sequence selected from the group consisting of:
NTFGSVIPATGPL
LYSANFESLIPANADPVVTTQNIIVTG
LYSANFEYLIPANADPVVTTQNIIVTG
PASGKMWIAGDG
EAGKKYTFTMRRA, and
EAGKKYHFLMKKM.

3. A composition as claimed in claim 1 in which the at least one peptide comprises a sequence selected from the group consisting of:

FLLDADHNTFGSVIPATGPLFTGTASS
LYSANFESLIPANADPVVTTQNIIVTG
LYSANFEYLIPANADPVVTTQNIIVTG
TNPEPASGKMWIAGDGGNQP
RYDDFTFEAGKKYTFTMRRAGMGDGTD
DDYVFEAGKKYHFLMKKMGSGDGTE, and
TNPEPASGKMWIAGDGGNQPARYDDFTFEAGKKYTFTMRRAGMGDGTD

PASGKMWIAGDG
EAGKKYTFTMRRA, and
EAGKKYHFLMKKM.

10. A peptide as claimed in claim 8 comprising at least one sequence selected from the group consisting of:
FLLDADHNTFGSVIPATGPLFTGTASS
LYSANFESLIPANADPVVTTQNIIVTG
LYSANFEYLIPANADPVVTTQNIIVTG
TNPEPASGKMWIAGDGGNQP
RYDDFTFEAGKKYTFTMRRAGMGDGTD
DDYVFEAGKKYHFLMKKMGSGDGTE, and
TNPEPASGKMWIAGDGGNQPARYDDFTFEAGKKYTFTMRRAGMGDGTD

11. A peptide as claimed in claim 8 in which the peptide is selected from the group consisting of:
FLLDADHNTFGSVIPATGPLFTGTASS
LYSANFESLIPANADPVVTTQNIIVTG
LYSANFEYLIPANADPVVTTQNIIVTG
TNPEPASGKMWIAGDGGNQP
RYDDFTFEAGKKYTFTMRRAGMGDGTD
DDYVFEAGKKYHFLMKKMGSGDGTE, and
TNPEPASGKMWIAGDGGNQPARYDDFTFEAGKKYTFTMRRAGMGDGTD

12. Use of a peptide as claimed in any one of claims 8 to 11 as an antigen in a diagnostic test.

13. An antibody specifically directed against a composition as claimed in any one of claims 1 to 7 or a peptide as claimed in any one of claims 8 to 11.

14. An antibody as claimed in claim 13 which is a monoclonal antibody.

15. Use of an antibody as claimed in claim 13 or claim 14 in a diagnostic test.

16. A composition comprising an antibody as claimed in claim 13 or claim 14 and a pharmaceutically acceptable carrier or diluent.

17. A method of reducing the prospect of *P. gingivalis* infection in an individual and/or severity of disease, the method comprising administering to the individual an amount of a composition as claimed in any one of claims 1 to 7 effective to induce an immune response in the individual directed against *P. Gingivalis*.

18. A method of reducing the prospect of *P. gingivalis* infection in an individual and/or severity of disease, the method comprising administering to the individual an effective amount of a composition as claimed in 16.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Dated this first day of March 1999

THE UNIVERSITY OF MELBOURNE,
VICTORIAN DAIRY INDUSTRY AUTHORITY
Patent Attorneys for the Applicant:

F B RICE & CO

Sequence: 2

Leu Tyr Ser Ala Asn Phe Glu Ser Leu Ile Pro Ala Asn Ala Asp Pro
1 5 10 15

Val Val Thr Thr Gln Asn Ile Ile Val Thr Gly
20 25

SEQ ID NO: 3

Length: 27

Type: PRT

Organism: Porphyromonas gingivalis

Sequence: 3

Leu Tyr Ser Ala Asn Phe Glu Tyr Leu Ile Pro Ala Asn Ala Asp Pro
1 5 10 15

Val Val Thr Thr Gln Asn Ile Ile Val Thr Gly
20 25

SEQ ID NO: 4

Length: 20

Type: PRT

Organism: Porphyromonas gingivalis

Sequence: 4

Thr Asn Pro Glu Pro Ala Ser Gly Lys Met Trp Ile Ala Gly Asp Gly
1 5 10 15

Gly Asn Gln Pro
20

Lys Tyr Thr Phe Thr Met Arg Arg Ala Gly Met Gly Asp Gly Thr Asp
35 40 45

SEQ ID NO: 8
Length: 13
Type: PRT
Organism: Porphyromonas gingivalis

Sequence: 8
Asn Thr Phe Gly Ser Val Ile Pro Ala Thr Gly Pro Leu
1 5 10

SEQ ID NO: 9
Length: 12
Type: PRT
Organism: Porphyromonas gingivalis

Sequence: 9
Pro Ala Ser Gly Lys Met Trp Ile Ala Gly Asp Gly
1 5 10

SEQ ID NO: 10
Length: 13
Type: PRT
Organism: Porphyromonas gingivalis
Sequence: 10
Glu Ala Gly Lys Lys Tyr Thr Phe Thr Met Arg Arg Ala
1 5 10

Arg Tyr Asp Asp Phe Thr Phe Glu Ala Gly Lys Lys Tyr Thr Phe Thr
115 120 125

Met Arg Arg Ala Gly Met Gly Asp Gly Thr Asp Met Glu Val Glu Asp
130 135 140

Asp Ser Pro Ala
145

SEQ ID NO: 13
Length: 13
Type: PRT
Organism: Porphyromonas gingivalis

Sequence: 13
Ala Asn Glu Ala Lys Val Val Leu Ala Ala Asp Asn Val
1 5 10

SEQ ID NO: 14
Length: 13
Type: PRT
Organism: Porphyromonas gingivalis

Sequence: 14
Leu Ala Ala Asp Asn Val Trp Asp Gly Asn Thr Gly Tyr
1 5 10

SEQ ID NO: 15
Length: 13
Type: PRT
Organism: Porphyromonas gingivalis

SEQ ID NO: 19
Length: 13
Type: PRT
Organism: Porphyromonas gingivalis

Sequence: 19
Thr Gly Thr Ala Ser Ser Asp Leu Tyr Ser Ala Asn Phe
1 5 10

SEQ ID NO: 20
Length: 13
Type: PRT
Organism: Porphyromonas gingivalis

Sequence: 20
Leu Tyr Ser Ala Asn Phe Glu Ser Leu Ile Pro Ala Asn
1 5 10

SEQ ID NO: 21
Length: 13
Type: PRT
Organism: Porphyromonas gingivalis

Sequence: 21
Ser Leu Ile Pro Ala Asn Ala Asp Pro Val Val Thr Thr
1 5 10

SEQ ID NO: 22
Length: 13
Type: PRT
Organism: Porphyromonas gingivalis

SEQ ID NO: 26
Length: 13
Type: PRT
Organism: Porphyromonas gingivalis

Sequence: 26
Gly Lys Met Trp Ile Ala Gly Asp Gly Gly Asn Gln Pro
1 5 10

SEQ ID NO: 27
Length: 13
Type: PRT
Organism: Porphyromonas gingivalis

Sequence: 27
Asp Gly Gly Asn Gln Pro Ala Arg Tyr Asp Asp Phe Thr
1 5 10

SEQ ID NO: 28
Length: 13
Type: PRT
Organism: Porphyromonas gingivalis

Sequence: 28
Arg Tyr Asp Asp Phe Thr Phe Glu Ala Gly Lys Lys Tyr
1 5 10

SEQ ID NO: 29
Length: 13
Type: PRT
Organism: Porphyromonas gingivalis

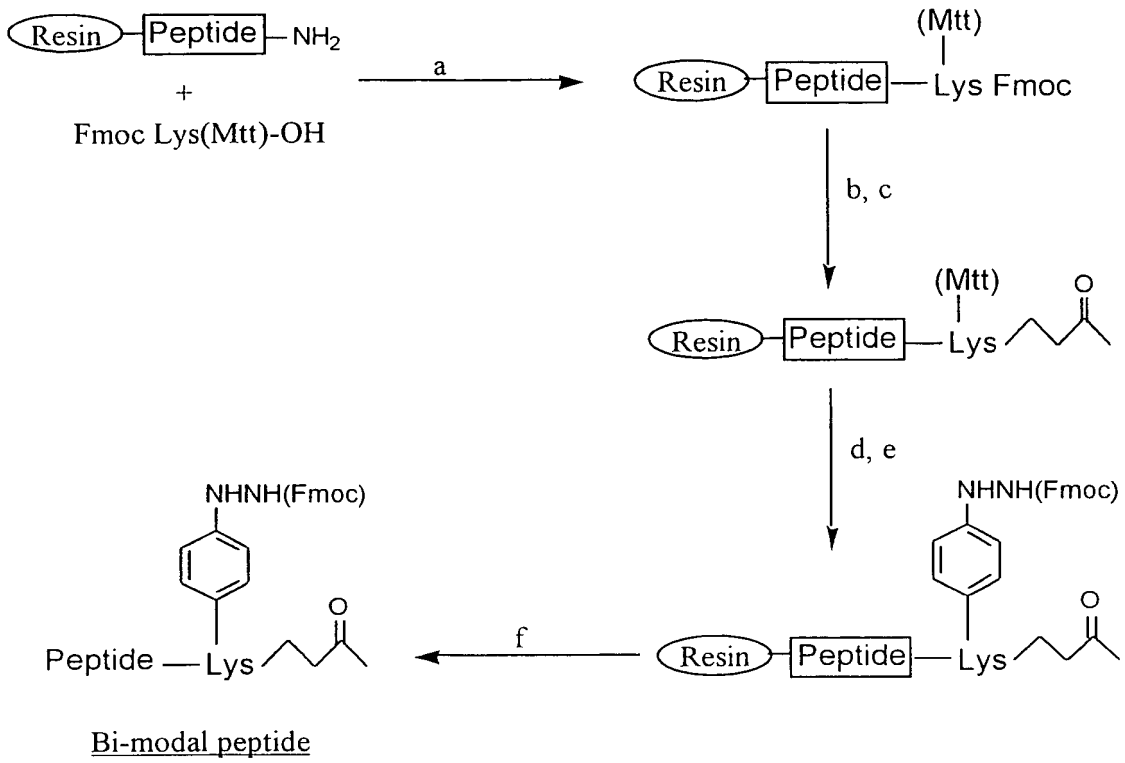
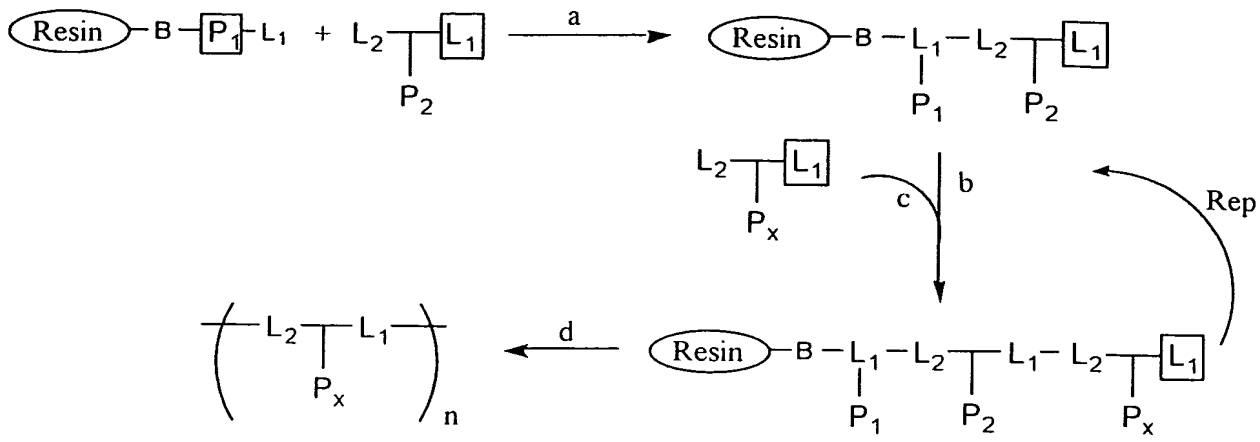
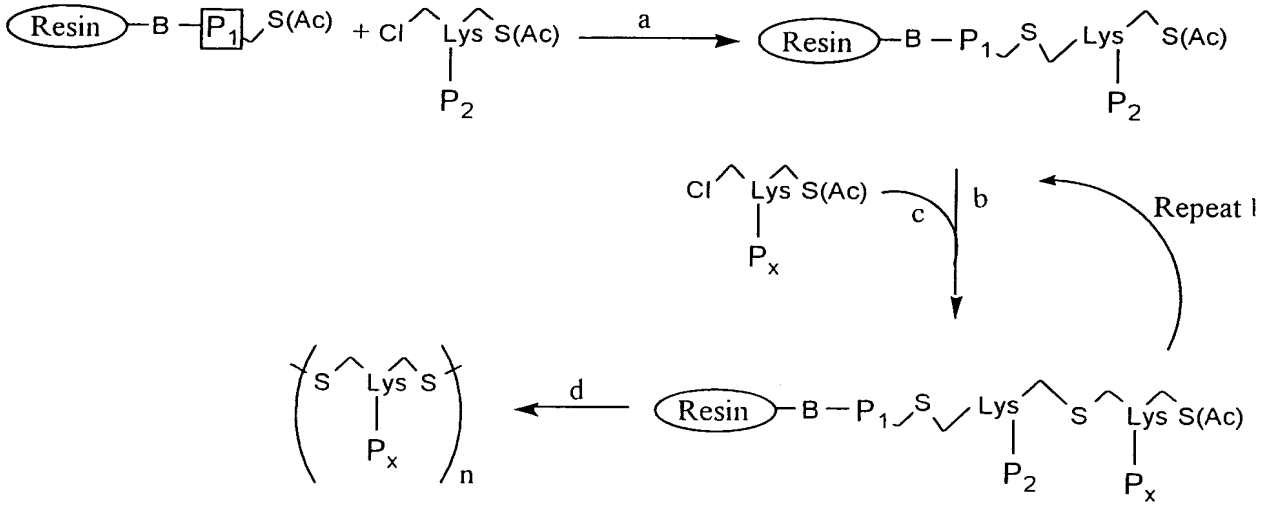

Resin
Peptide
NH2
+
Fmoc Lys(Mtt)-OH
a
(Mtt)
Resin
Peptide
Lys Fmoc
b, c
(Mtt)
Resin
Peptide
Lys
d, e
NHNH(Fmoc)
NHNH(Fmoc)
Peptide
Lys
f
Resin
Peptide
Lys
Bi-modal peptide

Figure 1

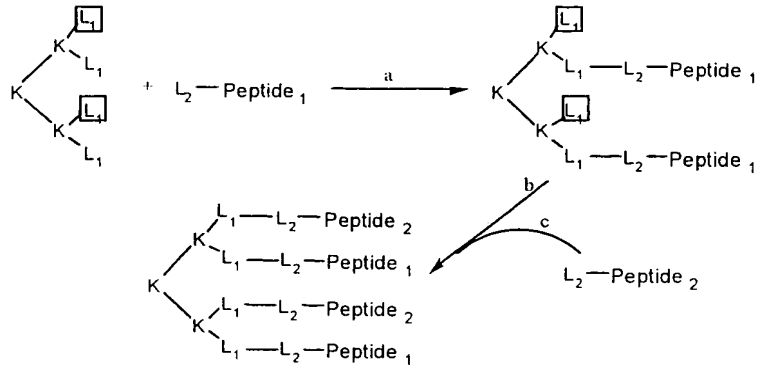


Example



5

Figure 3



Example

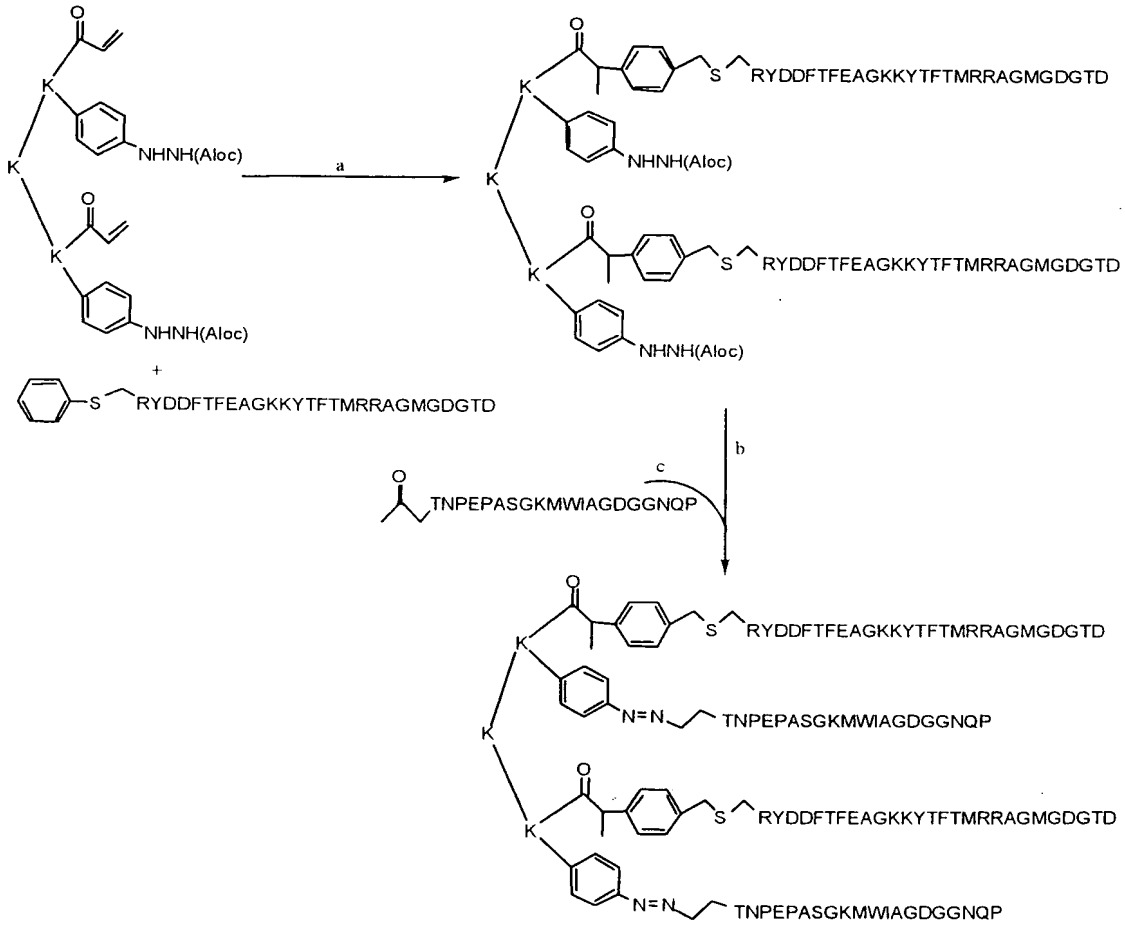


Figure 5

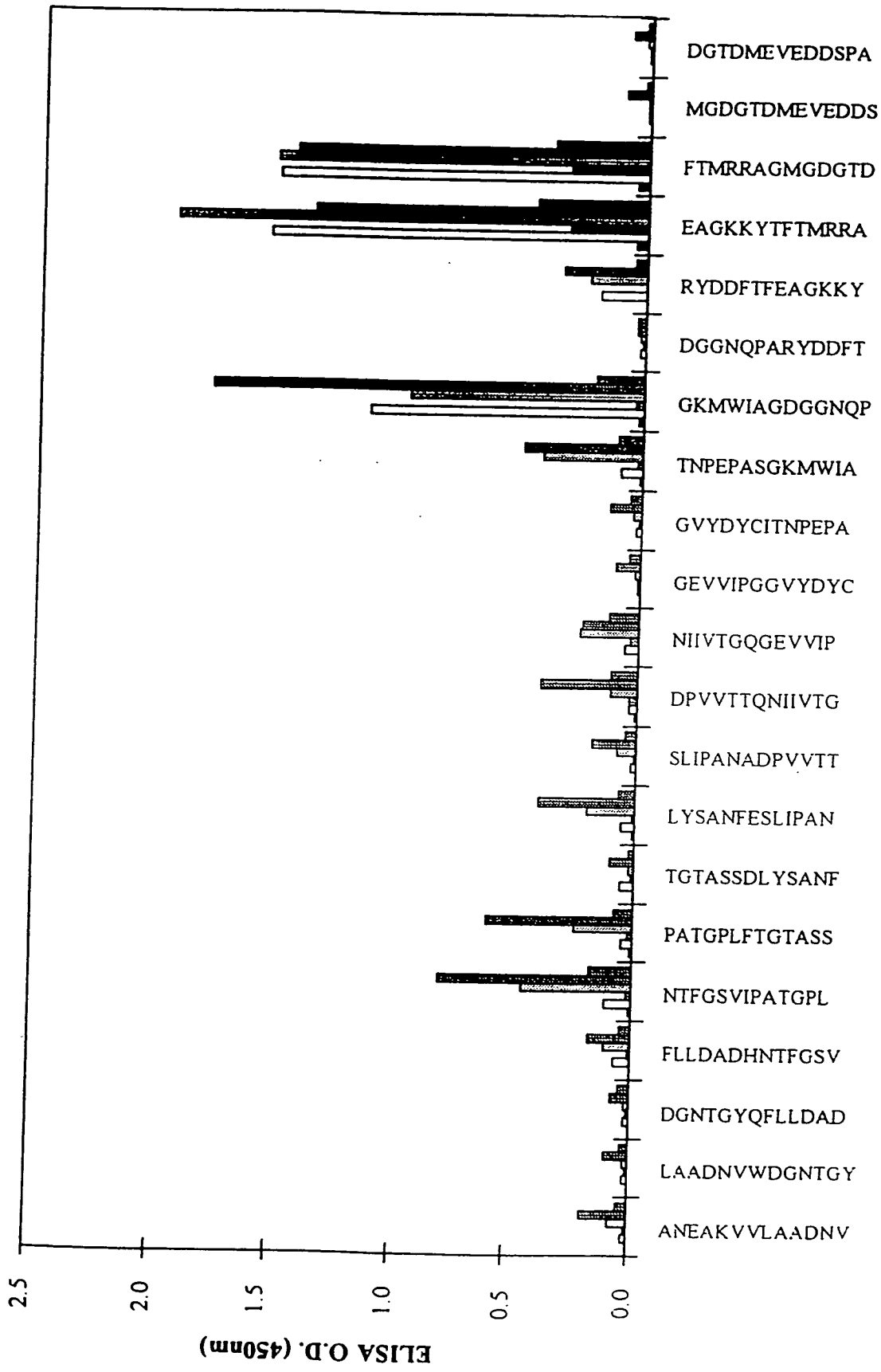


Figure 7: Serum IgG antibody responses assessed by ELISA to *Porphyromonas gingivalis PrtR-27* overlapping peptides. Twenty one PIN-bound peptides were probed with normal mouse sera (□), protective mouse sera (■), normal human serum (▩), patient D24 sera (▩), patient H10 sera (■) and patient D20 sera (■). ELISAs were developed as per Example 1.